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ON THE RELEASE OF TRANSMITTER AT NORMAL, MYASTHENIA GRAVIS AND MYASTHENIC SYNDROME AFFECTED HUMAN END-PLATES

BY S. G. CULL-CANDY*, R. MILEDI, A. TRAUTMANN† AND O. D. UCHITEL

From the Department of Biophysics, University College London, Gower Street, London WC1E 6BT

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SUMMARY

1. Transmitter release has been studied at normal, myasthenia gravis (m.g.) and myasthenic syndrome (m.s.) affected human end-plates. At normal and diseased end-plates evoked transmitter release is Poisson for a mean quantal content, m less than ten.

2. The relation between log m and log $[Ca]_o$, at normal and m.g. end-plates is linear, with a slope of 3·3–3·4. The value of m at m.g. end-plates is about five times larger than normal, below Ca 0·7 mM (Mg, 2 mM). This difference in m is reduced at higher Ca levels.

3. The slope of the relation between log m.e.p.p. frequency and log $[K]_o$ is similar at normal and m.g. end-plates. Over its linear portion the relationship has a slope of approximately 6.

4. Fluctuations in the latency of evoked transmitter release were compared at normal and m.g. nerve terminals. At normal end-plates the probability of release reaches a peak about 0·3–0·4 msec after unitary e.p.p.s of the shortest latency and returns to zero about 1·0 msec after the peak. At m.g. end-plates the distribution of latencies shows less uniformity.

5. At m.s. end-plates m is approximately 5 in normal Ringer solution (2 mM-Ca, 1 mM-Mg). The relation between log m and log $[Ca]_o$ is linear, with a slope of 1·0–1·5. The K dependence of m.e.p.p. frequency appears reduced at m.s. end-plates.

6. Assuming a co-operative mechanism for transmitter release at normal human motor nerve terminals, the dissociation constant for the Ca complex is about $1{\cdot}6 \times 10^{-3}$ M and the dissociation constant for the Mg complex is about $1{\cdot}0 \times 10^{-3}$ M.

7. It is concluded that the presynaptic changes, at m.g. end-plates, are not the primary cause of the defect in nerve muscle transmission. At m.s. end-plates the presynaptic changes are sufficient to account for failure in transmission. Possible mechanisms for the abnormalities in transmitter release are considered.

INTRODUCTION

In an attempt to understand more about the mechanism of transmitter release

* Beit Memorial Research Fellow.

† Present address: Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 Rue d'Ulm, 75005 Paris, France.

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from normal and diseased human motor nerve terminals we have compared transmitter release at normal end-plates with that at myasthenia gravis (m.g.) and Eaton–Lambert myasthenic syndrome (m.s.) affected end-plates in human muscle fibres.

At m.g. end-plates the defect in neuromuscular transmission has been suggested from recent studies to be primarily post-synaptic. Briefly, there is a reduction in size of the miniature end-plate potentials (m.e.p.p.s) (Elmqvist, Hofmann, Kugelberg & Quastel, 1964) and miniature end-plate currents (m.e.p.c.s) (Cull-Candy, Miledi & Trautmann, 1978; Cull-Candy, Miledi & Uchitel, 1979) and an accompanying loss of α-bungarotoxin binding sites (Fambrough, Drachmann & Satyamurti, 1973; Green, Miledi, Perez de la Mora & Vincent, 1975) and ACh sensitivity (Alberquerque, Rash, Mayer & Satterfield, 1976; Ito, Miledi, Vincent & Newsom Davis, 1978; Cull-Candy, Miledi & Trautmann, 1978). At Eaton–Lambert m.s. human end-plates, where the defect in neuromuscular transmission is apparently presynaptic in origin, there is a reduction in the number of transmitter packets released in response to a single nerve impulse whereas the post-synaptic response to a packet of transmitter appears normal (Lambert & Elmqvist, 1971).

This study is concerned with the possible occurrence of abnormalities in the transmitter release process in myasthenia gravis and myasthenic syndrome. A preliminary report on a part of this work has appeared (Cull-Candy, Miledi & Trautmann, 1978).

METHODS

Intercostal muscles were from patients clinically diagnosed to have myasthenia gravis, or myasthenic syndrome. These muscles were obtained either during thymectomy or as a biopsy. Control muscles were obtained during thoracotomy from patients without neuromuscular disease. All the m.g. patients had previously undergone anticholinesterase therapy.

Preparation. Muscles were dissected to give several preparations for electrophysiological study. They were investigated at 23 °C and remained in good condition for 2 days stored at 23° C. In experiments where end-plate potentials were studied nerve branches were dissected and sucked into a fine glass capillary electrode for stimulating.

Medium. Muscles were perfused continuously with an oxygenated (95% O_2/5 % CO_2) medium of the following composition (mM): NaCl, 113; Na_2HPO_4, 1·0; $NaHCO_3$, 25; KCl, 4·5; $CaCl_2$, 2·0; $MgSO_4$, 1·0; D-glucose, 11; pH 7·2. In many experiments where e.p.p.s were recorded the Mg concentration was doubled and the Ca concentration was varied as indicated in the experiments. Changes in Ca concentration were allowed a minimum equilibration time of 30–40 min. Changes in the K concentration were made by the addition of an appropriate concentration of K to the perfusing medium.

Neostigimine (10^{-6}–10^{-7} g/ml.) was usually added to the medium in experiments where transmitter release at m.g. end-plates was being investigated and in some of the experiments at normal and m.s. end-plates.

Estimation of quantal parameters. When comparing m.e.p.p. amplitudes in various fibres (see Table 1) allowance has been made for differences in resting potential, V_m, using the correction factor, m.e.p.p. $\times$ $80/V_m$ where the equilibrium potential for the transmitter is zero mV at the human end-plate (Cull-Candy, Miledi & Trautmann, 1979).

For calculating the mean quantal content, m, of the end-plate potential (e.p.p.) both m.e.p.p.s and e.p.p.s were recorded when possible. To obtain a reasonable number of m.e.p.p.s (the frequency being low at human end-plates) their frequency was often increased by a brief period of tetanic stimulation after sufficient e.p.p.s had been taken. A minimum of ten to twenty m.e.p.p.s was considered sufficient to give a reasonable value for m.e.p.p. amplitude provided they were well above the noise level. To calculate the standard deviation (S.D.) of e.p.p.s 50–100 records were taken. Records were rejected if there had been a change of $>$ 3 mV in the resting potential.

Mean quantal content was estimated using three methods (Katz, 1966; Martin, 1966) as specified in the results.

Failures method: $m_0 = \ln$ (number of impulses/number of failures)

Coefficient of variation: $m_1 = (\overline{\text{e.p.p.}}/\text{S.D.}_{\text{e.p.p.}})^2$

Direct method: $m_2 = (\overline{\text{e.p.p.}}/\overline{\text{m.e.p.p.}})$

In calculating m, non-linear summation was taken into account (Martin, 1966) when necessary.

RESULTS

Transmitter release from normal and myasthenia gravis nerve terminals

Assuming that Poisson's law applies to evoked transmitter release from human motor nerve terminals, when the probability of transmitter release is low (i.e. in low extracellular Ca and increased Mg), it is possible to predict the number of responses to nerve impulses which will contain 0, 1, 2, 3, . . . quantal components, i.e. n_0, n_1, n_2, n_3, . . . n_k where $n_0 = Ne^{-m}$, $n_k = (m/k)n_{k-1}$ (where N is the total number of trials and m is the mean quantal content). When a sufficiently accurate estimate of the mean m.e.p.p. amplitude ($\overline{\text{m.e.p.p.}}$) was available for myasthenic end-plates, m was obtained from $m_2 = \overline{\text{e.p.p.}}/\overline{\text{m.e.p.p.}}$. In the absence of an accurate value for $\overline{\text{m.e.p.p.}}$, it was possible to estimate the quantum size from $q = (\text{S.D.}_{\text{e.p.p.}})^2/\text{e.p.p.}$ (Katz, 1966; Martin, 1966). In all cases there was reasonable agreement between predicted and observed values of m at m.g. and m.s. end-plates at values of $m < 10$ (Fig. 1); typical results from these experiments are shown in Table 1.

TABLE 1. The number of e.p.p.s predicted by Poisson theorem to contain 0, 1, 2, 3, . . . quantal components (n_0, n_1, n_2, n_3, . . .) over the number of observed quantal components of e.p.p.s. For details see text.

Myasthenia gravis			Predicted/observed						
m.e.p.p. (mV)* (Corrected for RP = 80 mV)			n_0	n_1	n_2	n_3	n_4	n_5	n_6
F no. 1	0·22	$m = 1·1$	65/65	72/75	39/39	14/12	3/3	1/1	—
F no. 2	0·14	$m = 1·0$	34/33	34/35	17/15	6/6	2/3	0/1	—
F no. 3	0·40	$m = 1·75$	43/38	75/76	65/68	38/36	17/19	6/7	2/1
Myasthenic syndrome									
m.e.p.p. (mV)†									
F no. 4	0·80	$m = 0·84$	38/39	32/33	13/10	4/5	1/0	—	
F no. 5	0·57	$m = 1·44$	25/24	36/38	26/30	12/8	4/4	1/2	

* Prostigmine 10^{-6} g/ml. † No prostigmine.

Fig. 1 illustrates a comparison between direct estimates of the mean quantal content, m_2, with indirect estimates, m_1 (from the coefficient of variation of the e.p.p.), at end-plates where both estimates were obtained. Extracellular Ca concentrations were altered to produce a range of m values. The continuous line in Fig. 1 indicates perfect agreement between the two estimates. Quantal contents less than 8 fit reasonably to the continuous line. Of these two methods of estimating m only the

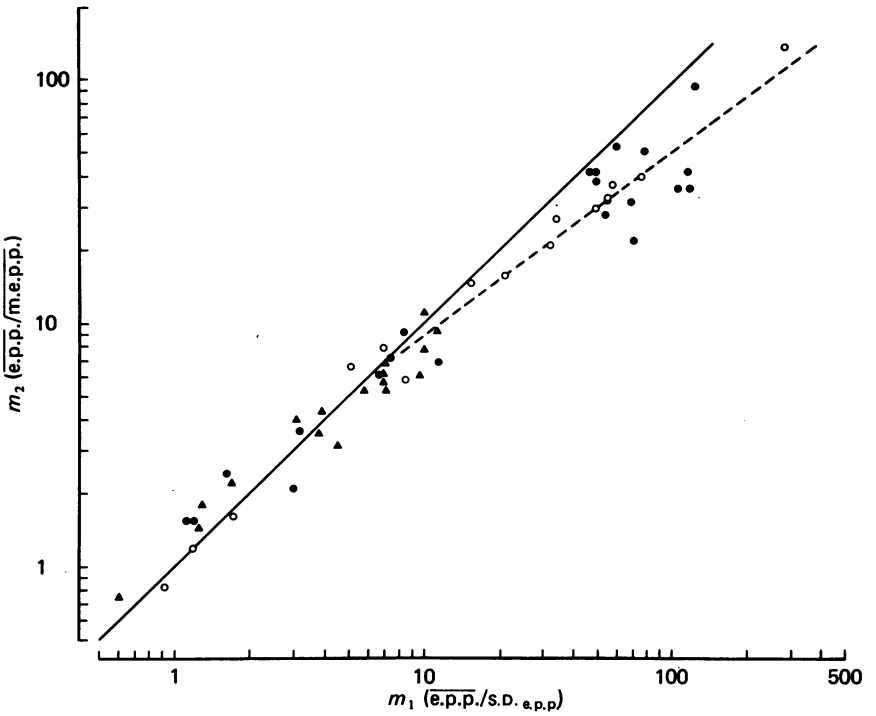

Fig. 1. Comparison of direct (m_2) and indirect (m_1) methods of estimating quantal content of e.p.p.s, plotted on double logarithmic co-ordinates. Values are shown for normal end-plates (●), m.g. end-plates (○) and m.s. end-plates (▲). Each symbol represents a single estimate (obtained from 50–100 e.p.p.s) at one end-plate. Values of m were obtained over a range of Ca concentrations 0·2–2·0 mM (2 mM-Mg). The continuous line corresponds to equality of the two estimates of m; the dashed line is a least squares fit to value of $m > 8$. The indirect method, which assumes a Poisson distribution of the quanta, is correct only for $m < 8$. This means that for $m > 8$, the probability of release is no longer small.

method of obtaining m_1 from the coefficient of variation assumes that transmitter release is Poisson. Agreement is therefore expected if transmitter release does indeed fit Poisson statistics (del Castillo & Katz, 1954; Martin, 1966). For values of m greater than approximately 8 there was a marked deviation of m from a Poisson distribution, as already shown for other species (del Castillo & Katz, 1954; Miyamoto, 1975). The indirect estimation of quantal content was larger than the actual quantal content obtained directly after non-linear summation had been corrected for. This difference is expected if the probability of release of a transmitter packet is not negligibly small and so follows a binomial rather than a Poisson distribution. In the cases where the number of measurements was large enough to give reasonable estimates of the transmitter release parameters, n and p (Martin, 1966), the observed distribution of e.p.p.s was a good fit to a binomial distribution. As this procedure was not possible for all estimations of m the dashed line in Fig. 1 is empirical. The relationship agrees well with that previously described for the frog (see Miyamoto, 1975), and allows a 'correction' to be made to large indirectly estimated values of

m when direct measurements were not possible. Thus indirect values of m, on the abscissa could be 'corrected' by reading the equivalent direct estimate on the ordinate.

Ca dependence of transmitter release at myasthenia gravis end-plates

The dependence of m on extracellular calcium, $[Ca]_o$, was examined at normal and m.g. human end-plates. Three methods were used to determine m (see Methods). To

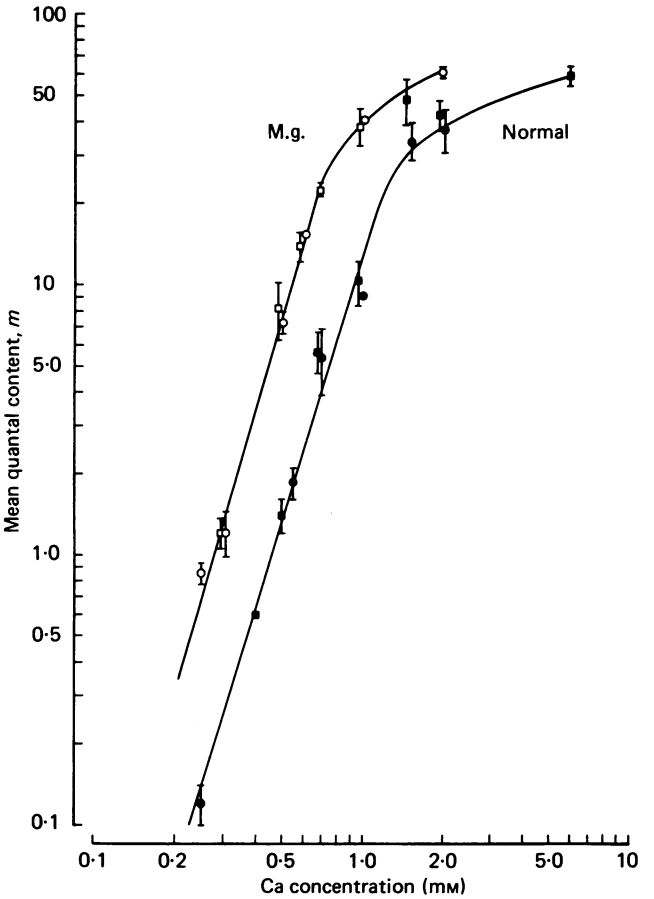

Fig. 2. Relationship between mean quantal content (m) of e.p.p.s and extracellular Ca concentration at normal (■, ●) and myasthenia gravis (m.g.; □, ○) end-plates plotted on double logarithmic co-ordinates. Filled and open circles represent *direct* estimations of m, $(\overline{e.p.p.}/\overline{m.e.p.p.})$; filled and open squares represents *indirect* estimations of m, $(\overline{e.p.p.}/s.d._{e.p.p.})^2$ which have been corrected using the method indicated in Fig. 1 (see text for details). The normal and the myasthenic curves were constructed from estimates obtained at twenty-seven end-plates (six muscles) and thirty-three end-plates (six muscles) respectively (50–100 e.p.p.s per estimation). All values are mean ± S.E. (shown only when larger than the size of the symbol). Straight lines through the points were fitted by the least squares method for values of $m < 20$ and have slopes of 3·3 for the normal end-plates and 3·4 for myasthenic end-plates. At low concentrations of Ca (0·25–0·7 mM) the quantal content at myasthenic end-plates is approximately five times higher than at normal end-plates whereas at higher concentrations this difference is reduced. 2 mM-Mg was present throughout; $T = 23$–25 °C.

obtain representative values of m four or five end-plates in a muscle were sampled at each Ca concentration. Fig. 2 shows the relation between m and $[Ca]_o$ constructed from estimates obtained at twenty-seven normal and thirty-three m.g. end-plates and plotted on logarithmic co-ordinates. At end-plates where the low frequency or small amplitude of m.e.p.p.s did not allow an accurate estimation of quantal size, m was estimated by an indirect method and then corrected (as described above) if $m > 10$. As can be seen in Fig. 2 good agreement was obtained between direct estimates (m_2) and corrected indirect estimates (m_1) at any given $[Ca]_o$ at normal and myasthenic end-plates.

The relationships fitted to the mean m values have slopes of 3·3 for normal end-plates and 3·4 for myasthenic end-plates when plotted on logarithmic co-ordinates. This compares with slope values of approximately 3·0 which were obtained when single human end-plates were followed over a range of three or four Ca concentrations (Cull-Candy, Miledi & Trautmann, 1978). In low $[Ca]_o$ the relation between log m and log $[Ca]_o$ is apparently linear; at higher $[Ca]_o$ the relationships for normal and m.g. end-plates deviate from linearity at approximately similar m values but at different Ca concentrations.

Although the shapes of the relationships between $[Ca]_o$ and quantal content were similar at normal and myasthenic end-plates the curve for myasthenic end-plates was displaced towards the lower Ca concentrations. The shift indicates that the mean number of quanta released per impulse is about five times larger for myasthenic nerve terminals at any given Ca concentration over the range 0·25–0·7 mM. The log of transmitter release as a function of log $[Ca]_o$ deviates from linearity above approximately 0·7 mM-Ca for myasthenic terminals and above approximately 1·0 mM-Ca for normal terminals. For this reason the difference between m values at myasthenic and normal end-plates becomes less apparent above 0·7 mM-Ca. For example, at 2 mM-Ca (2 mM-Mg) myasthenic terminals release approximately twice as many quanta per nerve impulse as normal. So far it has not been possible to decide whether a marked difference occurs in the levels of transmitter release at normal and m.g. end-plates in normal Ringer solution (2 mM-Ca, 1 mM-Mg). Indeed, it has previously been reported that there is no difference in m at normal and m.g. end-plates when recorded in normal Ringer solution (Elmqvist *et al.* 1964; Lindstrom & Lambert, 1978).

Several sources of error must be considered in estimating m in our experiments. Direct estimation of m_2 depends critically on an accurate measurement of m.e.p.p. amplitude. If the m.e.p.p.s are small, as is the case at m.g. end-plates, then the m.e.p.p.s measured will represent the upper range of the amplitudes and m will be underestimated.

When m, estimated from the coefficient of variation (m_1), was greater than about 10, it was corrected as described above. If the amplitude of the e.p.p. measured is small, the background noise contributes to the variance leading to an over-estimate of the quantum size, q, and an under-estimate of m. This would be expected to have a greater effect of the calculation of m at m.g. end-plates where the size of the e.p.p. is smaller than normal at any given level of extracellular Ca.

Frequency of miniature end-plate potentials

Ca dependence. M.e.p.p. frequency at normal and m.g. human end-plates increased with extracellular Ca concentration over the range 2–20 mM (2 mM-Mg). The slope of the relationship, m.e.p.p. frequency *vs.* [Ca], had a value of approximately 1·0 when plotted on logarithmic co-ordinates. However, variations between different samples

and the relatively small number of muscles available did not allow us to decide whether there were consistent differences between normal and m.g. end-plates in the m.e.p.p. frequencies over the range of $[Ca]_o$ levels studied.

Effect of potassium. To determine the influence of a steady depolarization of the nerve terminals on the m.e.p.p. frequency we have examined the effect of K at normal and m.g. end-plates. In the three m.g. samples used in this set of experiments the mean m.e.p.p. frequency in normal Ringer solution (4·5 mM-K) was higher than at normal human end-plates. However, this was not always the case. In other m.g. end-plates we have also encountered lower than normal m.e.p.p. frequency in 4·5 mM-K (see also Albuquerque *et al.* 1976). As the basal frequency of m.e.p.p.s was high in these particular preparations it is perhaps to be expected that the frequency would be high

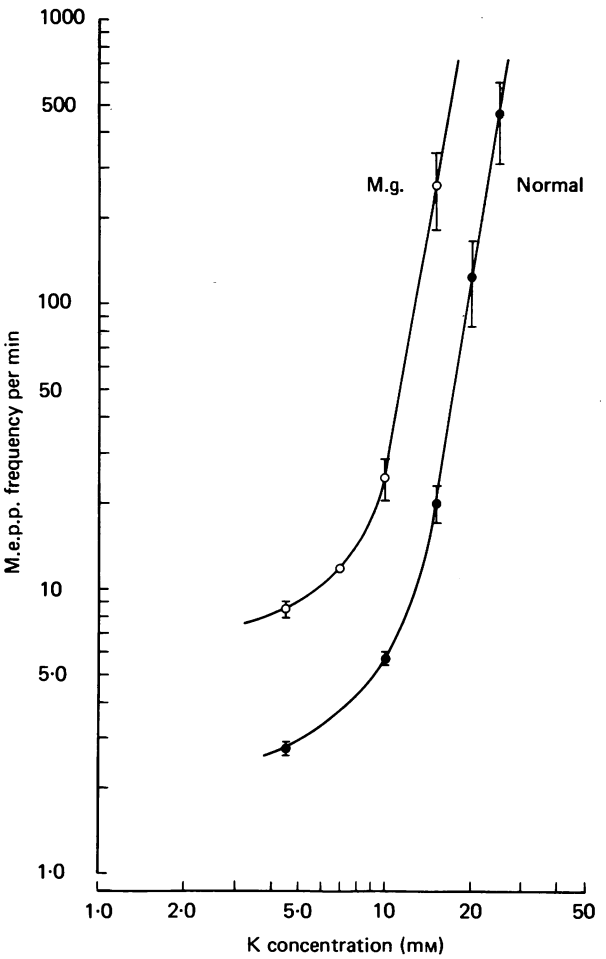

Fig. 3. Relation between m.e.p.p. frequency and extracellular K concentration at normal (●) and myasthenia gravis (m.g.; ○) end-plates plotted on logarithmic co-ordinates. Each point represents averaged data from at least fifteen end-plates in three muscles. Straight lines through the points (10–25 mM-$[K]_o$) have slopes of 6·1 for normal end-plates and 5·8 for m.g. end-plates. All values are mean ± S.E. (except for 7·0 mM where S.E. smaller than circle) 1 mM-Mg, 2 mM-Ca present throughout. $T = 23$–25 °C.

at all levels of K (Fig. 3). Therefore, a correlation between the absolute m.e.p.p. frequency for normal and m.g. end-plates, at the various K levels is not possible from these experiments. It remains to be seen whether there is a normal K dependence of the frequency at m.g. end-plates when the initial m.e.p.p. frequency is low.

According to the constant field theory (Hodgkin & Katz, 1949) at low concentrations K produces a small effect on the membrane potential; at higher $[K]_o$ the membrane potential decreases logarithmically as a function of $[K]_o$ as predicted by Nernst's equation. In Fig. 3 for $[K]_o$ above 10–15 mM there is an approximately linear relation between log $[K]_o$ and log m.e.p.p. frequency (see also Liley, 1956) and the relationships fitted to the mean values have slopes of 6·1 for normal end-plates and 5·8 for m.g. end-plates. The sensitivity of m.e.p.p. frequency to changes in $[K]_o$ is therefore similar at normal and m.g. end-plates.

Transmitter release by pairs of impulses at normal and m.g. end-plates

The effect of a conditioning nerve impulse on the ability of a subsequent impulse to release packets of transmitter was investigated at normal and myasthenic nerve terminals. The nerve was stimulated at 0·33 Hz with twin pulses and the interval between pulses was varied from 10 to 600 msec. More than twenty measurements were made at each interval.

In the presence of 2 mM-Mg and 1 mM-Ca, normal and myasthenic terminals showed facilitation of transmitter release for intervals up to 50–100 msec. For an interval of more than 100 msec the conditioning impulse produced no measurable effect on the release of transmitter by the second impulse. At this level of Ca or Mg the muscle did

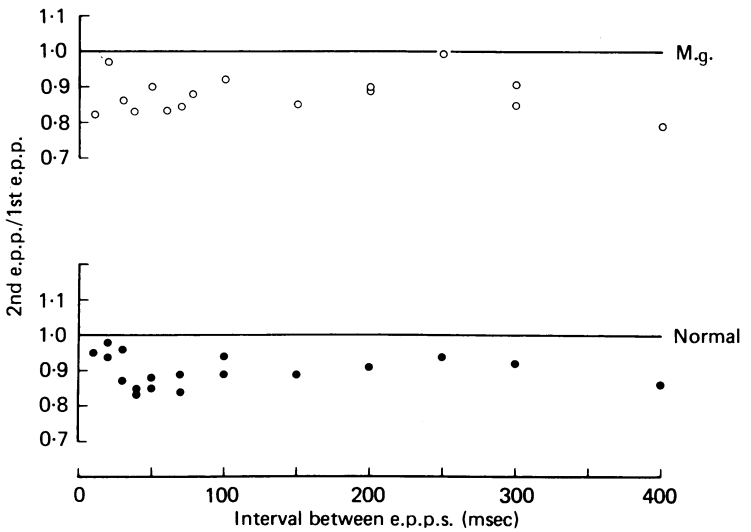

Fig. 4. 'Depression' of a second e.p.p. elicited within 10–400 msec of a preceding e.p.p. in normal Ringer solution (2 mM-Ca, 1 mM-Mg) at $T = 22$–24 °C at a normal end-plate (●) ($dTC = 2 \times 10^{-6}$ g/msec) and an m.g. end-plate (○). Ordinate is 'depression' defined as the ratio of the amplitudes: 2nd e.p.p./1st e.p.p.; abscissa is interval between the two e.p.p.s. Each point represents the average value from thirty pairs of e.p.p.s. For intervals of 10–400 msec the second e.p.p. is depressed by an average of 10 % compared with the first one at normal and myasthenic end-plates. For short intervals the amplitude of the second e.p.p. was measured from the extrapolated tail of the first e.p.p.

not twitch in response to the first or second nerve impulse. In the presence of 1 mM-Mg, 2 mM-Ca and 2×10^{-6} g/ml. (+)-tubocurarine at normal end-plates (i.e. when the quantal content was large) normal and myasthenic terminals showed a slight depression at all intervals up to 400 msec (see Fig. 4), as previously described for other mammalian end-plates (Thies, 1965).

At the Ca concentrations used, these experiments showed no apparent differences between normal and myasthenic nerve terminals in the ability of a second impulse to release transmitter. Therefore the 'fatigue' of transmission at m.g. end-plates may result from post-synaptic rather than presynaptic abnormalities: since the e.p.p. has a reduced amplitude in myasthenia gravis a further depression following a conditioning impulse or train of impulses would be sufficient to change the e.p.p. from supra- to sub-threshold.

Fluctuations in the latency of transmitter release

Following the arrival of an impulse at a nerve terminal there is a synaptic delay of less than 1 msec before the onset of the e.p.p. (Katz & Miledi, 1965). When the e.p.p. consists of unit potentials there is variation in the delay between the arrival of the nerve impulse and the appearance of the unit potentials. Frequency histograms of the delay indicate the time during which the probability of quantal release is increased.

To obtain a measure of the distribution of latencies between presynaptic spike and post-synaptic response, e.p.p.s composed of one or two units were recorded intracellularly at ten myasthenic and five normal end-plates. Evoked release of transmitter was reduced to a few units by high $[Mg]_o$ (2 mM) and low $[Ca]_o$ so that more than 50 % of impulses failed to release transmitter. Under these conditions most e.p.p.s are composed of a single quantum (del Castillo & Katz, 1954). Since the arrival of the impulse at a nerve terminal is practically constant after the stimulus, the fluctuations in the timing of release of single packets of transmitter were obtained by measuring the time interval between stimulus artifact and the foot of the e.p.p. (for 50–100 e.p.p.s). To eliminate small differences which may occur in the time taken by nerve impulses to reach the various end-plates the results have been pooled by taking the shortest delay at each end-plate as zero time. Fig. 5 shows histograms constructed from data obtained at five normal and five m.g. end-plates.

At normal human end-plates the probability of release reaches a peak about 0·3–0·4 msec after unitary e.p.p.s of the shortest time interval. Many units occur later than this time, but their probability of occurrence returns to zero at about 1·5 msec. When compared with normal, the histograms for myasthenic end-plates not only reach the peak value more slowly but the peak is broader. This was the case in all except one of the myasthenic end-plates.

Transmitter release from myasthenic syndrome nerve terminals

In four patients with Eaton–Lambert myasthenic syndrome (m.s.), the mean amplitude of the m.e.p.p.s was similar to those obtained at normal human end-plates. However, stimulation of the nerve produced subthreshold e.p.p.s at all end-plates tested due to a reduction in m (see Lambert & Elmqvist, 1971; Lindstrom & Lambert, 1978). The values for m were obtained in normal Ringer solution (2 mM-Ca,

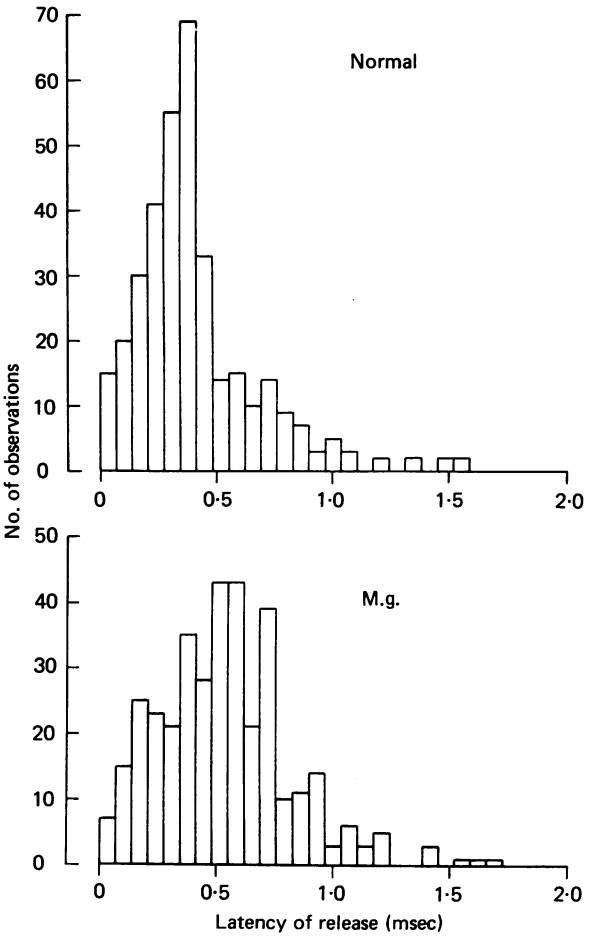

Fig. 5. Fluctuations in the latency of evoked release of single packets of transmitter at human end-plates. Normal end-plates: data pooled from five end-plates (351 'single-packet e.p.p.s'). Myasthenic end-plates: data pooled from five end-plates (358 'single-packet e.p.p.s'). 'Single-packet e.p.p.s' were recorded intracellularly from end-plates which showed > 50 % failure of impulses to release transmitter (in low extracellular Ca). The distribution of latencies was obtained by measuring the time delay between the stimulus artifact and the foot of the e.p.p. Abscissa is the distribution in msec where time zero is taken as the shortest delay at each end-plate. $T = 23$ °C. See text for further details.

1 mM-Mg) in biopsies from three patients. In one muscle $m = 6{\cdot}2 \pm 1{\cdot}0$ (mean ± S.E.; n = five end-plates); in a second muscle $m = 5{\cdot}09 \pm 1{\cdot}26$ (n = three end-plates); in a third muscle biopsy one bundle gave $m = 4{\cdot}25 \pm 1{\cdot}6$ (n = four end-plates); a second bundle from the same muscle studied after being maintained for 24 hr in normal Ringer solution gave $m = 5{\cdot}89 \pm 1{\cdot}45$ (n = four end-plates). This compares with a value for m of 50–60 at normal human end-plates under similar conditions (Elmqvist *et al.* 1964; Lindstrom & Lambert, 1978). At seventeen m.s. end-plates, where m was estimated from $\overline{\text{e.p.p.}}/\overline{\text{m.e.p.p.}}$ and from $(\overline{\text{e.p.p.}}/\text{S.D.}_{\text{e.p.p.}})^2$ (see Fig. 1), reasonably

good correlation was found between the two estimates of m over the range of values studied. This indicates that transmitter release was described well by Poisson statistics over the range of extracellular Ca concentrations 1·0–2·0 mM where the maximum m value was about 10. Thus the probability of release is abnormally low at these end-plates.

Although not systematically studied, marked facilitation of e.p.p.s was obtained in normal Ringer solution in response to repetitive nerve stimulation (Elmqvist & Lambert, 1968).

Ca and Mg dependence of transmitter release at m.s. end-plates

Ca dependence. As shown in Fig. 6 the mean quantal content, m of the e.p.p. at m.s. end-plates depends on the extracellular Ca concentration. The Ca sensitivity of the transmitter release process at m.s. terminals is lower than that of normal terminals. Therefore it was not possible to study transmitter release in m.s. end-plates at the Ca and Mg concentrations where the relation log m *vs.* log $[Ca]_o$ was linear at normal

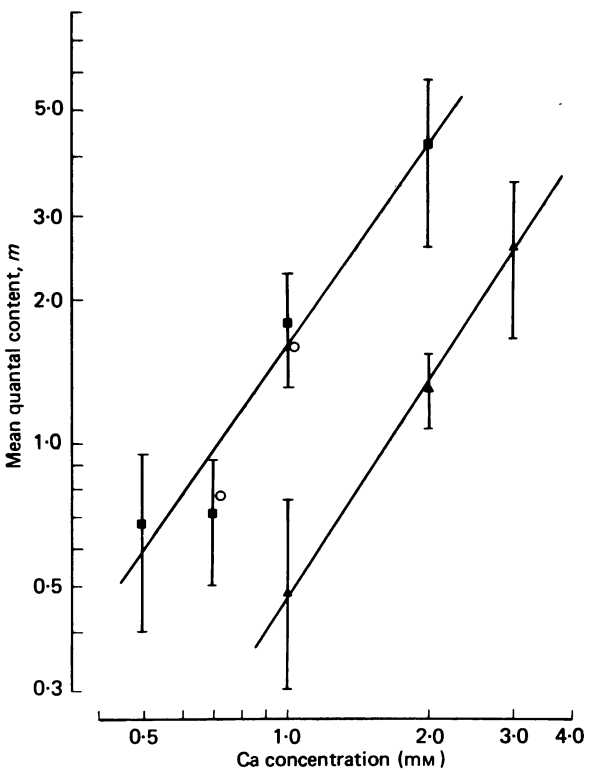

Fig. 6. Relation between mean quantal content, m, and extracellular Ca concentration at myasthenic syndrome end-plates. The large values of the S.E. bars reflects variability of m from end-plate to end-plate in myasthenic syndrome. Straight lines through the points are fitted by the least squares method.

Two relationships obtained from a single muscle. ■, 1 mM-Mg, regression line slope = 1·43; curve constructed from estimates of m at forty-one end-plates. ▲, 2 mM-Mg, regression line slope = 1·55; curve constructed from estimates of m at twelve end-plates. ○, 0 mM-Mg. T = 23–25 °C.

end-plates. However, the two types of end-plate were compared over the same range of quantal content values at which transmitter release is a Poisson process.

End-plates were investigated in the presence of 0, 1 or 2 mM-Mg. Fig. 6 shows the relationship m *vs.* $[Ca]_o$ plotted on logarithmic co-ordinates. Transmitter release differed in two ways from release at normal terminals. The value for m was at least one order of magnitude less than that at normal human end-plates studied under similar conditions (see Fig. 2). In addition, transmitter release had a low sensitivity to changes in the extracellular Ca concentration when compared with normal terminals over the same range of m values. This is reflected in the slope of the relationship which for the three bundles studied was in the range 1·0–1·5 compared with 3·3 at normal end-plates. It seems unlikely that the reduced slope of the relationship at m.s. end-plates resulted from sampling over a non-linear part of the log m *vs.* log $[Ca]_o$ relationship even though at the levels of $[Ca]_o$ used the relationship at normal end-plates is saturating. Thus although results are scattered, the data in Fig. 6 give no evidence of a non-linear trend. In addition, the release process at this level is Poisson which indicates that the number of available packets is not being exhausted, although saturation in some other part of the release process, which might give rise to non-linearity, cannot be excluded. Evidence that the relationship was relatively linear over the range of Ca concentration studied was also obtained in experiments where the Mg dependence of transmitter release was investigated.

Mg dependence. Fig. 6 shows examples of the relation between Ca concentration and m obtained at three Mg concentrations in preparations from one muscle sample. A decrease in $[Mg]_o$, from 2 to 1 mM causes the curve to shift to the left in a parallel manner. However, the slope of the curve remains the same, indicating that the relationship log m *vs.* log $[Ca]_o$ is being examined over its linear portion. No further shift in the curve is obtained when $[Mg]_o$ is decreased from 1 to 0 mM. The Mg sensitivity of m at m.s. human end-plates is consistent with that previously reported for rat end-plates (Hubbard, Jones & Landau, 1968*b*).

Effect of potassium on m.e.p.p. frequency

Fig. 7 shows the relation between log $[K]_o$ and log m.e.p.p. frequency at m.s. end-plates (in a preparation from the muscle sample used in the experiments illustrated in Fig. 6). For comparison, the relationship m.e.p.p. frequency *vs.* $[K]_o$ for normal human end-plates is represented by a dashed line. Over the linear portion of the curve (i.e. 15–25 mM-$[K]_o$) the relationship for m.s. end-plates has a slope of 4·6. At normal end-plates the slope of the relationship is about 6. Although it has been possible to study the relationship m.e.p.p. frequency *vs.* $[K]_o$ in only one m.s. muscle sample, the reduced effect of $[K]_o$ at m.s. end-plates is in agreement with an earlier report where end-plates were studied at 32 °C (Lambert & Elmqvist, 1971). In the m.s. sample used in these experiments the m.e.p.p. frequency in 4·5 mM-K (see Fig. 7) is slightly higher than the frequency at normal end-plates and at m.s. end-plates in some of the other muscle samples studied. No conclusions about the absolute m.e.p.p. frequencies can be drawn from this observation in a single muscle especially as good agreement has previously been found between normal and m.s. end-plates (Elmqvist & Lambert, 1968). As a result of the initial elevation of the m.s. m.e.p.p. frequency, combined with a reduced sensitivity to $[K]_o$, the normal control m.e.p.p. frequency

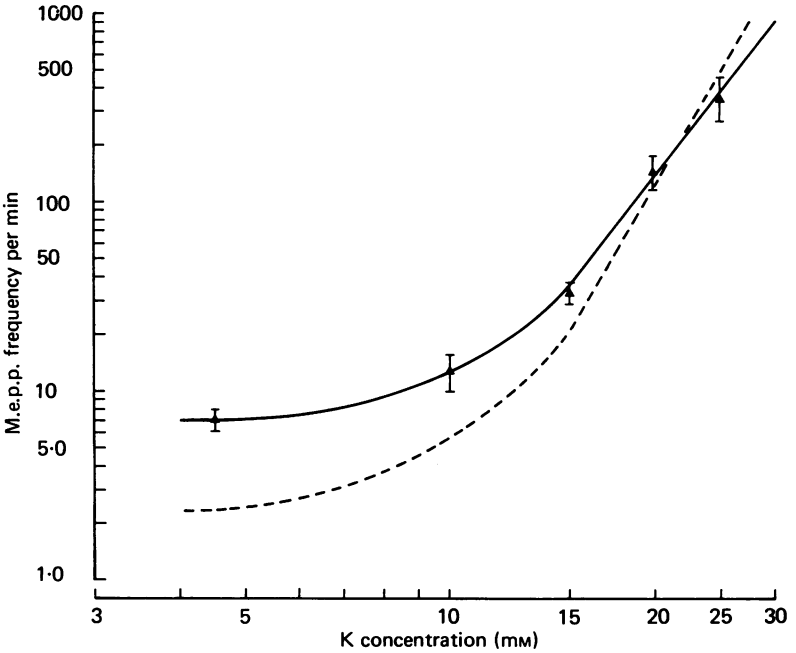

Fig. 7. Relation between m.e.p.p. frequency and extracellular K concentration at myasthenic syndrome end-plates plotted on logarithmic co-ordinates. Each point represents averaged data from at least six end-plates in one muscle. Straight line through the points (15–25 mM-$[K]_o$) has a slope of 4·6. All values are mean ± S.E. For comparison, the dashed line shows the relationship obtained in normal human end-plates (see Fig. 3). 1 mM-Mg, 2 mM-Ca present throughout $T = 23$–25 °C.

begins to exceed that at these m.s. end-plates only at K levels above 25 mM. Biochemical measurements of ACh release in this muscle sample showed that release evoked by 50 mM-K was indeed reduced when compared to normal control values (P. C. Molenaar & R. L. Polak, personal communication).

Ca and Mg action at normal nerve terminals

Assuming that in nerve terminals two parallel reactions occur which involve the combination of a divalent cation with an active site, X (del Castillo & Katz, 1954) and that K_1 is the dissociation constant for CaX and K_2 is the dissociation constant for MgX, it is possible to explain the power relation between log m and log $[Ca]_o$ as a co-operative interaction of Ca ions in the release of a single packet of ACh (Jenkinson, 1957; Dodge & Rahamimoff, 1967). Then, in terms of such a model, at the normal and m.g. human end-plate a co-operative action of at least three or four CaX complexes would be required. If Mg competes with Ca for the active site it is possible to derive the dissociation constants. K_2 was obtained by comparing log m *vs.* $[Ca]_o$ curves in the presence of 5 mM and 2 mM-$[Mg]_o$ and K_1 was obtained directly by plotting $m^{-1/3}$ against $[Ca]_o^{-1}$ as previously described in detail (see Dodge & Rahamimoff, 1967; Balnave & Gage, 1973). Data were pooled from twenty-eight normal end-plates in the presence of 5 mM-Mg and forty normal end-plates in the presence of 2 mM-Mg; the dissociation constant for the Ca complex, K_1, is approximately $1{\cdot}6 \times 10^{-3}$ M and the

dissociation constant for the Mg complex, K_2, is approximately $1{\cdot}0 \times 10^{-3}$ M (calculated for $m = 2$).

DISCUSSION

Impulse-evoked release of transmitter from normal human nerve terminals and from m.g. and m.s. nerve terminals is reasonably well described by Poisson statistics up to a quantal content of about 10, indicating that the probability of release of any one packet of transmitter is low (see del Castillo & Katz, 1954; Martin, 1966; Katz, 1966; Lambert & Elmqvist, 1971).

Increased transmitter release at myasthenia gravis end-plates. One of our main findings was that m.g. motor nerve terminals released about 2–5 times as many packets of transmitter as normal nerve terminals at all Ca levels examined. Although the value of m at the higher Ca concentrations is somewhat uncertain it seems that one of the factors which determine the level of transmitter release is abnormal in m.g. terminals. It is unclear whether the abnormality involves a change in the level of extracellular Ca at which saturation of transmitter release occurs or a change in the maximum number of transmitter packets released.

It is necessary to consider possible artifacts which could be involved in such differences in transmitter release. Consistent differences in the treatment of the two groups of nerve–muscle samples may have arisen during their removal from patients. This could, for example, have led to one group of muscles being kept for a short period under anoxic conditions. In addition m.g. muscles were from patients who had received anticholinesterase therapy, although long term treatment of rats with neostigmine reduces rather than increases the number of transmitter packets released by a nerve impulse (Roberts & Thesleff, 1969). Finally the normal and m.g. muscle samples are lateral intercostal and parasternal intercostal muscles respectively. It is feasible that the quantal content of the e.p.p.s in these two muscles could show systematic differences, although probably not of the magnitude described here.

Assuming that the differences between the two types of nerve terminal are real rather than artifactual in origin then the greater fluctuation in the latency of transmitter release together with the larger number of transmitter packets released by a nerve impulse arriving at the m.s. nerve terminal could have several possible causes. A nerve terminal action potential of longer than normal duration could produce a slower rise and decay in the probability of transmitter release and account also for an increase in m. A change in the latency histograms has indeed been shown when the duration of the pre-terminal spike is experimentally altered at frog end-plates (Katz & Miledi, 1967*a*, *b*; Benoit & Mambrini, 1970). The similarity of the sensitivity of m.e.p.p. frequency to K at normal and m.g. terminals is not inconsistent with this concept, although in this respect it would be of interest to study the relation between m.e.p.p. frequency and K concentration at m.g. terminals when the basal m.e.p.p. frequency is less than normal.

The increase in evoked transmitter release at m.g. terminals seems to correlate with biochemical studies which have shown an increased ACh content of m.g. human muscles (Ito, Miledi, Molenaar, Vincent, Polak, Van Gelder & Newsom Davis, 1976). It may therefore be that increased transmitter release reflects a higher than normal

level of ACh in the nerve terminals. However, the extra ACh in myasthenia, although largely located in end-plate containing segments of the muscle, need not necessarily be located in the nerve terminals. It may be relevant that KCl initially releases a higher than normal level of ACh (measured by mass spectroscopy) from myasthenic muscle, although this difference is abolished in tetrodotoxin (Ito *et al.* 1976; Molenaar, Polak, Miledi, Alema, Vincent, Newsom Davis, 1979).

In view of the positive correlation between m and the size of the end-plate in frog muscle (Kuno, Turkanis & Weakly, 1971) it is attractive to suppose that biochemical and physiological changes may both reflect a common process, namely an increase in presynaptic terminal area at m.g. end-plates. Such an increase in the nerve terminal size would explain the increase in the mean quantal content, and might also explain the less synchronous release of transmitter packets. There are several reports of morphological changes at m.g. end-plates indicating a prolongation of nerve terminals (Coërs & Telerman-Toppet, 1976), although the cross-sectional area of the terminal may be somewhat reduced (Engel & Santa, 1971).

Other explanations which could increase the level of transmitter release have not been discounted. Briefly, these include a variety of mechanisms which would result in an elevated level of intracellular free Ca in the pre-terminal, such as changes in Ca buffering capacity of the terminal or a greater than normal Ca influx due to changes in the pre-terminal membrane or its microenvironment.

Myasthenic syndrome end-plates. The process of transmitter release by nerve impulses at Eaton–Lambert m.s. end-plates has a reduced sensitivity to extracellular Ca. This is apparent from the reduced Ca dependence of the quantal content of the e.p.p. Thus over the range of values studied, transmitter release increased with a 1·0–1·5 power of the $[Ca]_o$ in contrast with normal end-plates where m is a function of the 3rd power of $[Ca]_o$. The power relation of log m *vs.* log $[Ca]_o$ at m.s. end-plates is similar to that previously described for frog end-plates where the e.p.p. is of very low quantal content (Crawford, 1974) and at botulinum-poisoned nerve terminals of rat (Cull-Candy, Lundh & Thesleff, 1976).

Impulse-evoked release of transmitter packets at m.s. terminals is a Poisson process and in three muscle samples studied fairly high levels of m.e.p.p.s were evoked in response to prolonged depolarization of nerve terminals by K. In addition biochemical studies have shown that the ACh content of these muscles is not reduced (Molenaar *et al.* 1979). Thus it seems that the low quantal content of e.p.p.s does not reflect a marked reduction in the size of the transmitter pool.

From what is known about the functioning of the squid giant synapse a simple decrease in the amplitude of the nerve terminal action potential would reduce the number of packets released. However, it would not account for the reduced dependence of the e.p.p. on $[Ca]_o$ in m.s. terminals (Katz & Miledi, 1970). In addition a reduced influx across the nerve terminal during the action potential, for example by blocking Ca channels with cobalt, does not alter the co-operativity of the calcium mechanism of release over the range of m values seen here (Crawford, 1974). Finally, the reduced sensitivity of the m.e.p.p. frequency to K indicates that the defect is still observed when transmitter release is produced by prolonged nerve terminal depolarization rather than by an action potential.

Kinetics of transmitter release at human end-plates. At normal and m.g. end-plates

the relationship log m *vs.* log Ca has a slope of 3·3–3·4. This compares with previous estimates of 3–5 for frog and toad (Dodge & Rahamimoff, 1967; Balnave & Gage, 1973; Dennis & Miledi, 1974), 2·7 for rat (Hubbard, Jones & Landau, 1968*b*; Cull-Candy *et al.* 1976), 4 for mouse (Cooke, Okamoto & Quastel, 1973) and 2·7 for the squid giant synapse (Katz & Miledi, 1970). At human end-plates a co-operative interaction of 3 or 4 Ca ions could explain the approximate 3rd power relationship of log m *vs.* log $[Ca]_o$, as suggested for transmitter release in other species (Jenkinson, 1957; Dodge & Rahmimoff, 1967; and see Discussion of Balnave & Gage, 1973). There could, of course, be other explanations for such a 3rd power relationship.

In terms of a co-operative model at the human end-plate the dissociation constants K_1 for the Ca complex and K_2 for the Mg complex have values around 1×10^{-3} M which suggests that the active site of the human nerve terminal has an affinity for Ca and Mg which is in the same range as that found in amphibians (Jenkinson, 1957; Dodge & Rahamimoff, 1967; Balnave & Gage, 1973; Crawford, 1974). If transmitter release at human end-plates is explained by a reaction of the type

$$3\,Ca + X \rightleftharpoons Ca_3X \overset{\alpha}{\rightharpoonup} \text{release}$$

then the release of transmitter from m.s. nerve terminals may result from a similar but non-co-operative reaction

$$Ca + X \rightleftharpoons CaX \overset{\beta}{\rightharpoonup} \text{release}$$

CaX would then be assumed to be less effective than Ca_3X at releasing a packet of transmitter (i.e. $\alpha > \beta$). A model has previously been proposed (Hubbard, Jones & Landau, 1968*a*) in which CaX, Ca_2X and Ca_3X are all capable of releasing transmitter packets to varying degrees and are formed as part of a sequential reaction of the form, $CaX \rightleftharpoons Ca_2X \rightleftharpoons Ca_3X$. Whether CaX is envisaged as forming in a sequential reaction or in a separate parallel reaction it could be that at m.s. end-plates the dominant complex releasing transmitter is CaX. Other complexes (e.g. Ca_2X, Ca_3X) may either not be formed or not release transmitter packets as a result of pathological changes in the terminal.

We conclude that there are presynaptic changes at both m.g. and Eaton–Lambert m.s. end-plates. The presynaptic changes at m.s. end-plates would be sufficient to account for failure of neuromuscular transmission (see also Lambert & Elmqvist, 1971). On the other hand, at m.g. end-plates the presynaptic changes are not the primary cause of the defect and would be expected to result in an increased rather than decreased efficiency of nerve–muscle transmission. The presynaptic changes at m.g. end-plates could therefore be a compensating mechanism resulting perhaps secondarily from the reduced efficiency of ACh on the post-synaptic membrane in myasthenia gravis or from the effect of anti-ACh receptor antibodies on presynaptic ACh receptors.

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